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Toxicity of Creosote-Contaminated Sediment to Field-and Laboratory-Colonized Estuarine Benthic Communities

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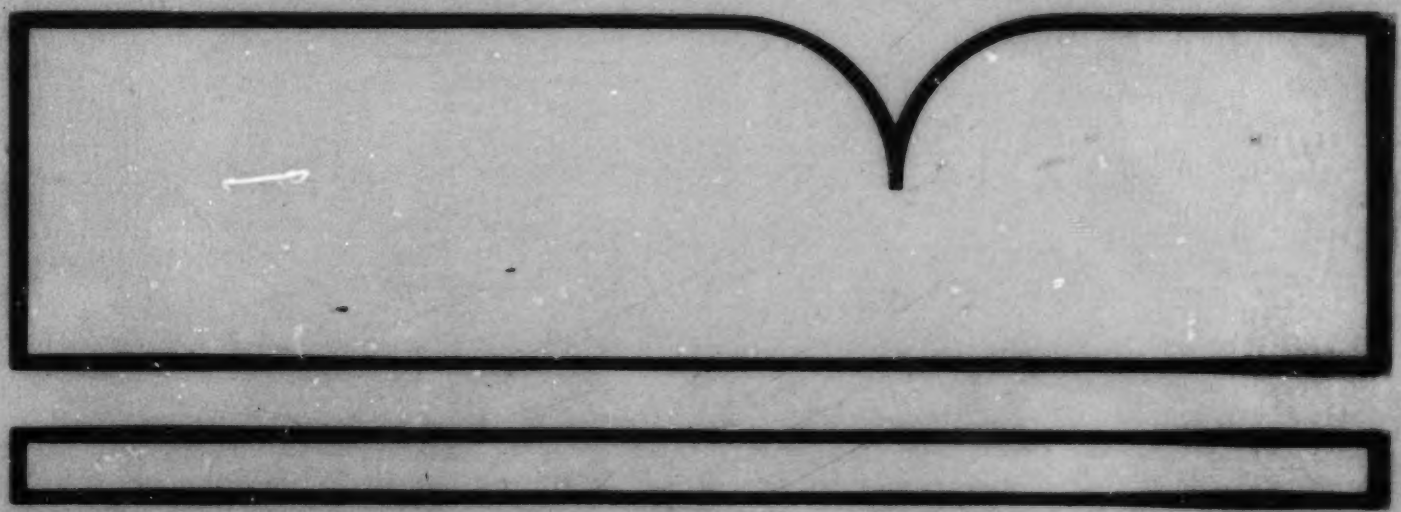

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16. ABSTRACT

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Key Words: Creosote, benthic communities, macrobenthos, toxicity, aquatic pollution.

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JOURNAL ARTICLE

TOXICITY OF CREOSOTE-CONTAMINATED SEDIMENT TO FIELD- AND LABORATORY-COLONIZED ESTUARINE BENTHIC COMMUNITIES

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Abstract — Macrobenthic animal communities that colonized uncontaminated and creosote-contaminated sand (177, 844 and 4,420 μg/g, nominal) during 8 weeks were compared to assess effects of marine-grade creosote on community structure. Aquaria were colonized in the laboratory by planktonic larvae entrained in continuously supplied unfiltered seawater and in the field by animals that occurred naturally. Individuals and species in aquaria that contained 844 and 4,420 μg creosote/g sand were significantly fewer ($\alpha = 0.05$) than those in the control. Abundance of animals in field-colonized communities contaminated with 177 μg/g creosote, but not in laboratory-colonized communities, also was less than that in the control. The lowest creosote concentration at either site that affected numbers of individuals or species was 844 μg/g for mollusks and 177 μg/g for echinoderms, annelids and arthropods. The Shannon-Weaver index of diversity, Simpson's index of dominance and the Bray-Curtis dissimilarity index differences were greater with each increase in creosote concentration. Rarefaction indices of diversity indicated that the distribution of individuals within species was about the same for laboratory and field assemblages of animals. Initial measured concentrations of creosote in sand (midrange concentration) decreased by 30% in the laboratory and by 42% in the field at the end of the 8-week test.

Keywords — Creosote Benthic communities Macrobenthos Toxicity Aquatic pollution

INTRODUCTION

Creosote is widely used to protect wood from attack by fungi, marine borers and insects. It is a distillate derived from coal tar produced by carbonization (coking) of coal, and consists chiefly of liquid and solid aromatic hydrocarbons.

Pollution of marine waters by creosote could result from processing, storage, transportation, wood treatment and use of treated marine pilings and foundation timbers. Much of the creosote produced in the United States is shipped by barge to storage facilities located on or near major river routes [1]. The most significant environmental input is from wood-treatment plant wastewater [2]. Although we know of no major discharge of creosote in the marine environment, the marketing of it is similar to that of petroleum products that have been involved in many of these discharges. A review of the literature on marine areas polluted with petroleum hydrocarbons shows that while concentrations dissolved in water were generally < 10 ppb, they ranged from 1,500 to 5,700 ppm in coastal sediments [3]. Currently, there is public concern that groundwater contamination from creosote sludge in industrial waste ponds

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might pollute Pensacola Bay, FL, 550 m from the site of those ponds. U.S. Geological Survey investigators have found the contaminant in streams tributary to the Bay and have expressed concern because its effect on bottom organisms is unknown [4].

Borthwick and Patrick [2] found that marine-grade creosote in water was moderately toxic to various estuarine organisms. They calculated 96-h EC_{50} values (mg/L) in static or flowing-water tests of 0.71 for oysters (*Crassostrea virginica*), 0.018 for mysids (*Mysidopsis bahia*), 0.24 for pink shrimp (*Penaeus duorarum*) and 0.72 for sheepshead minnows (*Cyprinodon variegatus*).

Mollusks and crustaceans also can accumulate creosote hydrocarbons. Neff and Anderson [5] reviewed findings that indicated that various bivalve mollusks and the northern lobster *Homarus americanus* were heavily contaminated with benzo[a]pyrene from creosote-treated pilings or timbers.

To obtain additional information on effects of marine-grade creosote on the estuarine environment, we conducted studies on responses of laboratory- and field-colonized macrobenthic communities exposed to the compound in sediment. Benthic animals can be particularly susceptible, since creosote is heavier than water and readily adsorbs to sediment particles.

METHODS AND MATERIALS

Experimental communities

Effects of creosote-contaminated sand on macrobenthic animals that colonized sand-filled aquaria for 8 weeks were determined by comparing community structures in contaminated and noncontaminated aquaria. Laboratory aquaria were colonized (June 28 to August 23, 1982) by settling of planktonic larvae supplied by continuously flowing unfiltered seawater from Santa Rosa Sound, FL. Field aquaria, placed in 3 m of water at a site in Santa Rosa Sound adjacent to our laboratory, were colonized (July 6 to August 31, 1982) by naturally occurring animals. Salinity and temperature of seawater entering the laboratory, recorded continuously, averaged 27.5 parts per thousand (20.5 to 33.5 ppt) and 29°C (26 to 31°C) during colonization.

Eight aquaria were used for the control and for each of three exposure concentrations of creosote for the laboratory and the field-colonized communities. The eight aquaria for each treatment consisted of four 32 cm × 32 cm × 6 cm deep boxes, divided diagonally in half and grouped in squares of four boxes (Fig. 1). Acrylic plastic was used in construction.

Aquaria were filled with clean silica sand (the control) or sand mixed with varying amounts of creosote. Sand (82% of particles were 0.36 to 0.71 mm) was dredged some months previously from Santa Rosa Sound. We used marine-grade creosote (specific gravity 1.015) from the same stock [supplied by U.S. Environmental Protection Agency (EPA) Pesticides Laboratory, Beltsville, MD] as that used in earlier studies at our laboratory [2]. Creosote (1, 5 or 25 ml) dissolved in 100 ml acetone was mixed by hand with 3,400 cc of sand for each aquarium. An equal volume of acetone (100 ml) was blended into control aquaria. Nominal concentrations of creosote were 0, 177, 884 and 4,420 μg/g; measured concentrations were 0, 105, 995 and 3,584 μg/g. Data are presented as nominal concentrations or as nominal concentrations designated as low (177 μg/g), medium (884 μg/g) and high (4,420 μg/g).

In the laboratory, groups of four boxes were placed in each of four box frames (Fig. 1) that, when filled, contained a 3-cm head of flowing seawater over the sand. Unfiltered seawater was delivered continuously to a splitter box, where four adjacent glass tubes supplied water at 1.5 L/min to the center of each frame. Water flowed from the frame through notched openings on all sides. Photoperiod was 12 h light:12 h dark; light was supplied by four 1.2-m fluorescent lamps (34 w). Eight days after the start of the laboratory study, scuba divers placed 16

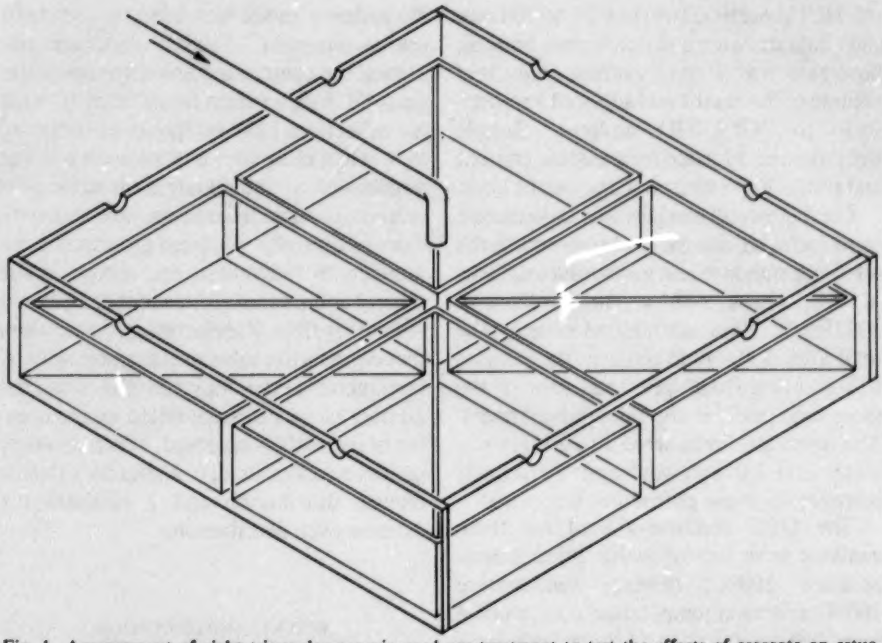

Fig. 1. Arrangement of eight triangular aquaria used per treatment to test the effects of creosote on estuarine macrobenthic communities. Each group of laboratory aquaria was placed in a frame, and seawater was continuously supplied at 1.5 L/min to the center.

boxes in four groups of four in the field. The boxes were positioned in the substratum so that their surfaces were level with the surrounding sand.

Weekly core samples taken with a modified 50-cc syringe (opening enlarged to full diameter) from medium-concentration (884 μg/g) aquaria in the laboratory and field were analyzed to determine any reduction in the amount of creosote during the 8-week study. To avoid disturbing the integrity of the benthic communities, these samples were taken from an additional group of four boxes at each site that were maintained under the same experimental conditions, but used only for sediment samples. In addition, water samples were taken weekly in the laboratory from the control and from all creosote-exposed aquaria and analyzed for creosote that leached from the sand.

Sediment oxidation-reduction potential (Eh) was determined for laboratory-developed communities at 2.5 and 8 weeks. Measurements were taken at 1-cm intervals in two control aquaria and in two aquaria of each concentration with a saturated calomel half-cell as a reference and a platinum indicating electrode. The electrodes were calibrated with a standard quinhydrone solution.

After 8 weeks of colonization, laboratory and field communities (covered with acrylic plastic and transferred to the laboratory) were harvested. Animals retained by a 1-mm mesh sieve were preserved, identified and counted.

Chemical analysis

For analysis of creosote in seawater, 1-liter water samples were acidified to red litmus and extracted with two 100-ml portions of 50% ethyl ether in petroleum ether (v/v). The extract was concentrated to approximately 20 ml with a steam bath, followed by nitrogen evaporation to not less than 1 ml for GLC analysis.

For analysis of creosote in sand, 5 to 10 grams of wet sand were extracted with 50 ml

1% HCl in acetone (v/v) in a 25 × 500 mm glass column with a sintered-glass bottom; flow rate was 1 to 3 ml/min. The final volume of the eluant was adjusted appropriately for GLC-FID analysis. Sample weights were adjusted for moisture content and results expressed on a dry weight basis.

Creosote in all samples was quantitated, using technical marine-grade creosote as the reference standard and measuring total area of nine peaks with a Hewlett-Packard 3353E laboratory automation system. The total area of the nine peaks in the samples was compared with the total area of the same nine peaks in the reference standard. The detection limits were 10 μg/L for seawater and 1.0 μg/g for sand. Percentage recovery for these procedures was >90.

The GLC conditions used for these analyses were the following: injector temperature 200°C, detector temperature 300°C and oven temperature programmed from 160 to 300°C at 16°/min with an 8-min hol d time at the end of the program. A 2-mm inner diameter × 1.3 m glass column packed with 60/80 mesh Tenax gave the most quantifiable chromatograms when helium was used as carrier gas at a flow rate of 30 ml/min. Flow rates of the detector gases were 30 ml/min for hydrogen and 240 ml/min for air.

Statistical analysis

Various comparisons and biological indices of community structure were applied to our laboratory and field-derived data. One-way analysis of variance and Duncan's *post hoc* analysis [6] were used to compare numbers of individuals and species in control and contaminated aquaria by site and by phylum ($\alpha = 0.05$). The Shannon-Weaver [7] index (H', $\log_{10}$) was calculated to assess differences in species diversity, an integrated measure of richness and evenness. Simpson's [8] index was used as a comparative measure of dominance; degree of dominance by the more abundant species determines the evenness of distribution of individuals among species. The Bray-Curtis dissimilarity index was used to document spatial–temporal faunal heterogeneity among the control and creosote concentrations [9]. Index values range from 1, when the collections have no species in common, to 0, when all species are common and the distribution of individuals is the same on a percentage basis. In addition, rarefaction indices of diversity [10] were generated from numbers of individuals and species in the control and treated groups for the field and laboratory data. Rarefaction is a procedure that corrects for unbalanced sample sizes. A rarefaction curve indicates the expected number of species represented by the number of individuals observed. When diversity indices are calculated, 0 implies the extreme uneven distribution and 1 indicates the extreme even distribution.

RESULTS AND DISCUSSION

Initial measured concentrations of creosote in sand (midrange concentration of 995 μg/g) decreased at the end of the 8-week test by 30% in the laboratory (693 μg/g) and by 42% in the field (575 μg/g). Decreases first occurred during the first week in the field and during the second week in the laboratory.

Creosote occurred in water samples from laboratory-developed communities on the 2nd d of exposure: 35 μg/L in the low concentration, 158 μg/L in the medium concentration and 510 μg/L in the high concentration. None was detected in the low and medium concentrations on the 9th d. Water samples from the high concentration contained 330 μg creosote/L on the 9th d, 50 μg/L on the 16th d and none on the 23rd d.

Because of limited leaching of creosote into the water column and a greater persistence in sediment, contaminated sediment may present a greater hazard to benthic organisms than water-borne exposure. Mysid (*Mysidopsis bahia*) bioassays and chemical analyses estimated the half-life of this same

marine-grade creosote in seawater to be < 1 week [2].

Redox potentials in laboratory aquaria differed among the control and various concentrations of creosote (Fig. 2). At 2.5 weeks, high Eh readings (>271 mv) indicated that the entire 6 cm of sediment in control aquaria was well oxidized and contained little organic matter. Profiles of the 0- to 3-cm layer of contaminated aquaria exhibited a similar pattern, but Eh values decreased moderately below this depth (>210 mv). At 8 weeks, Eh decreased with increasing depth in the control aquaria, particularly in the contaminated aquaria. Reduction was most pronounced in the high concentration, at which Eh decreased to negative values at depths below 4 cm. The pattern of decreasing Eh with increasing depth is similar to the redox profile reported for natural sandy substrates elsewhere [11–13]. Many complex biological and chemical transformations are involved when oxidizing processes become displaced by reducing processes [14], but creosote contamination resulted in a more anaerobic and chemically reducing environment than in the control.

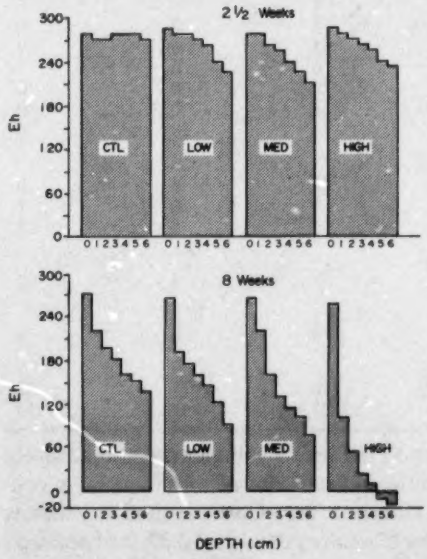

Fig. 2. Laboratory Eh profiles (mv) in the control (CTL) aquaria (sand) and in aquaria containing creosote-contaminated sand of low (177 μg/g), medium (MED) (844 μg/g) and high (4,420 μg/g) concentration. Each value is an average of two measurements at each depth (cm). 0 depth refers to overlying water.

A total of 367 animals representing 33 species of five phyla was collected from laboratory-colonized aquaria (Table 1). The average numbers of animals and species in aquaria containing 177 μg/g creosote were not significantly different from those in control aquaria. However, significantly fewer animals and species occurred in aquaria containing 844 or 4,420 μg/g than in the control (Table 2). Mollusca, Annelida, and Echinodermata were the dominant phyla. The lowest concentration that affected numbers of individuals or species was 177 μg/g for echinoderms, 844 μg/g for annelids and 4,420 μg/g for mollusks.

In field-colonized aquaria, 875 animals representing 60 species of 10 phyla were harvested (Table 3). Individuals and species averaged fewer in all concentrations of creosote than in the control (Table 4). Mollusks, annelids and arthropods were the dominant phyla. Mollusks were significantly affected by concentrations of 844 and 4,420 μg/g, annelids and arthropods by all concentrations. Although too few were collected to indicate statistical significance, the chordate *Branchiostoma caribaeum* may also have been affected by creosote: zero to four individuals occurred among the three concentrations, whereas there were 16 in the control.

Some differences in creosote toxicity between laboratory and field may have been due to differences in species sensitivity rather than to site. However, for animals like *Laevicardium mortoni*, one of the dominant mollusks at both sites, species specificity was not a factor.

Species diversity (H′) and dominance (Simpson's index) were similar for the control and low concentration (177 μg/g), but were less for the medium (884 μg/g) and high (4,420 μg/g) concentrations. Diversity indices for laboratory data were 1.09, 1.10, 0.81 and 0.68 for control, low, medium and high

Table 1. Animals in laboratory-colonized benthic communities collected from control aquaria and from aquaria with creosote-contaminated sand for 8 weeks (replicates were pooled)

Taxon	Control	Creosote (μg/g) 177	884	4,420
Mollusca				
Laevicardium mortoni	25	26	36	30
Acteocina canaliculata	12	21	5	4
Lyonsia hyalina	6	10	14	2
Diastoma varium	12	6	2	3
Abra aequalis	1	9	3	3
Musculus lateralis	3	2	2	0
Chione latilirata	1	0	1	0
Tagelus divisus	0	2	0	0
Tellina lineata	1	0	1	0
Anadora notabilis	1	0	0	0
Anomalocardia auberiana	0	1	0	0
Atrina rigida	1	0	0	0
Crassostrea virginica	1	0	0	0
Total mollusks	64	77	64	42
Annelida				
Neanthes succinea	3	3	3	3
Capitella capitata	8	1	0	0
Mediomastus californiensis	4	4	1	0
Polydora socialis	2	2	3	0
Armandia agilis	3	1	2	0
Prionospio heterobranchia	3	1	1	0
Hydroides dianthus	1	1	0	1
Pectinaria gouldii	1	2	0	0
Piromis sp.	1	2	0	0
Diopatra cuprea	0	0	0	2
Lumbrineris sp.	1	1	0	0
Capitellides jonesi	0	1	0	0
Megalomma bioculatum	0	1	0	0
Sabella micropthalma	1	0	0	0
Unidentified Phyllodocidae	1	0	0	0
Unidentified Syllidae	0	1	0	0
Total annelids	29	21	10	6
Echinodermata				
Leptosynapta inhaerens	32	5	0	0
Amphipholis squamata	0	0	0	1
Total echinoderms	32	5	0	1
Chordata				
Molgula manhattensis	2	7	3	3
Arthropoda				
Corophium acherusicum	1	0	0	0
Total all phyla				
Individuals	128	110	77	52
Species	26	23	14	10

concentrations, respectively. Those for field data were 1.18, 1.13, 0.91 and 0.70. Indices of dominance for laboratory communities were 0.88, 0.89, 0.75 and 0.66 for control, low, medium and high concentrations respectively. Those for field communities were 0.88, 0.88, 0.82 and 0.76.

The number of species in common to both contaminated aquaria and the control, and their relative abundance, progressively differed from control with increase in concentration. Bray-Curtis dissimilarity indices for laboratory data were 0.39, 0.51 and 0.58 for low, medium and high concentrations, in that order; those for field data were 0.51, 0.59 and 0.69.

Rarefaction indices of diversity also indicated that the average numbers of species in

Table 2. Average density of animals and average number of species (SEM) per aquarium collected from laboratory-colonized aquaria

Phylum	Control	Creosote (μg/g) 177	844	4,420
Mollusca	8.0 (0.9)	9.6 (1.0)	8.0 (0.8)	5.3 (1.8)
	4.1 (0.4)	4.1 (0.5)	3.4 (0.5)	2.0[a] (0.5)
Annelida	3.6 (0.4)	2.6 (0.6)	1.3[a] (0.3)	0.8[a] (0.3)
	3.0 (0.3)	2.4 (0.4)	1.3[a] (0.3)	0.8[a] (0.3)
Echinodermata	4.0 (1.3)	0.6[a] (0.4)	0[a] (0)	0.1[a] (0.1)
	0.8 (0.2)	0.3 (0.2)	0 (0)	0.1 (0.1)
All phyla	16.0 (1.8)	13.8 (1.2)	9.6[a] (1.1)	6.5[a] (1.8)
	8.3 (0.8)	7.1 (0.7)	4.9[a] (0.7)	3.1[a] (0.5)

[a]Significantly different from control ($\alpha = 0.05$).

Table 3. Animals in field-colonized benthic communities collected from control aquaria and from aquaria with creosote-contaminated sand for 8 weeks (replicates were pooled)

Taxon	Control	Creosote (μg/g) 177	884	4,420
Mollusca				
Crepidula maculosa	65	63	28	24
Marginella apicina	18	25	36	25
Laevicardium mortoni	33	14	8	3
Nassarius vibex	4	4	1	0
Brachidontes exustus	5	1	1	0
Crassostrea virginica	6	0	0	0
Mangelia stellata	0	1	3	1
Anomalocardia auberiana	0	0	3	1
Acteocina canaliculata	2	0	1	0
Musculus lateralis	2	1	0	0
Mitrella lunata	1	1	0	0
Abra lioica	0	1	0	0
Caecum nitidum	1	0	0	0
Diastoma varium	0	1	0	0
Lyonsia hyalina	1	0	0	0
Olivella mutica	1	0	0	0
Parastarte triquetra	1	0	0	0
Tellina sp.	1	0	0	0
Turbonella sp.	0	1	0	0
Total mollusks	141	113	81	54
Annelida				
Poecilochaetus johnsoni	129	11	0	0
Hydroides dianthus	19	18	14	16
Neanthes succinea	17	11	7	5
Notomastus latericeus	34	0	0	0
Polydora socialis	1	13	0	0
Capitella capitata	0	9	0	0
Prionospio heterobranchia	2	5	0	0
Axiothella mucosa	1	3	0	0
Haploscoloplos robustus	3	0	0	0
Pomatoceros sp.	1	0	1	0
Unidentified Oweniidae	0	1	1	0
Chone sp.	0	1	0	0
Diopatra cuprea	0	0	1	0
Gyptis vittata	1	0	0	0
Nereis sp.	1	0	0	0
Polycirrus sp.	0	1	0	0
Scolelepis sp.	1	0	0	0
Scoloplos rubra	1	0	0	0

Table 3. (*Continued*)

Unidentified Polynoidae	1	0	0	0
Unidentified Syllidae	0	0	1	0
Total annelids	212	73	25	21
Arthropoda				
Upogebia affinis	27	9	2	0
Grandidierella bonnieroides	9	5	2	2
Acanthohaustorius sp.	17	0	0	0
Pinnixa sp.	14	0	0	0
Listriella sp.	7	3	0	0
Tanaidacea (family Apseudidae)	10	0	0	0
Pagurus annulipes	4	1	0	0
Callinectes sapidus	3	0	0	0
Ampelisca sp.	1	0	0	0
Bowmaniella sp.	1	0	0	0
Edotea montosa	0	1	0	0
Processa hemphilli	1	0	0	0
Sicyonia laevigata	0	1	0	0
Total arthropods	94	20	4	2
Chordata				
Branchiostoma caribaeum	16	4	2	0
Rhynchocoela				
Unidentified no. 1	2	0	0	0
Unidentified no. 2	0	1	1	0
Total nemerteans	2	1	1	0
Echinodermata				
Ophiophragmus filograneus	0	3	0	0
Phoronida				
Phoronis architecta	2	0	0	0
Platyhelminthes				
Stylochus ellipticus	0	0	1	1
Cnidaria				
Unidentified Actiniaria	1	0	0	0
Unidentified Sipuncula	1	0	0	0
Total all phyla				
Individuals	469	214	114	78
Species	44	30	19	9

Table 4. Average density of animals and average number of species (SEM) per aquarium collected from field-colonized aquaria

		Creosote (μg/g)		
Phylum	Control	177	844	4,420
Mollusca	17.6 (2.7)	14.1 (1.6)	10.1[a] (1.2)	6.8[a] (0.9)
	5.3 (0.6)	4.3 (0.5)	3.5[a] (0.3)	2.6[a] (0.3)
Annelida	26.5 (2.3)	9.1[a] (2.1)	3.1[a] (0.5)	2.6[a] (0.7)
	5.1 (0.8)	4.1 (0.8)	2.0[a] (0.3)	1.3[a] (0.3)
Arthropoda	11.8 (2.0)	2.5[a] (0.7)	0.5[a] (0.3)	0.3[a] (0.2)
	5.3 (0.4)	1.8[a] (0.5)	0.4[a] (0.2)	0.3[a] (0.2)
Chordata	2.0 (0.9)	0.5 (0.3)	0.3 (0.2)	0 (0)
	0.6 (0.2)	0.4 (0.2)	0.3 (0.2)	0 (0)
All phyla	58.6 (3.7)	26.7[a] (4.3)	14.1[a] (1.7)	9.8[a] (1.3)
	16.9 (1.4)	10.9[a] (1.4)	6.4[a] (0.7)	4.3[a] (0.5)

[a]Significantly different from control ($\alpha = 0.05$).

aquaria that contained 844 and 4,420 μg/g creosote were significantly less ($\alpha = 0.05$) than those in control aquaria for both laboratory and field-colonized communities (Fig. 3).

Generally, there were more species for a given number of individuals in the field communities than in the laboratory communities, but the distribution of individuals within species was about the same (Fig. 3). However, only 13 species occurred in both laboratory and field (39% of the species in laboratory communities and 22% of those in field communities). The dominant mollusk and annelid in laboratory collections were third in abundance for these phyla in field collections. Concepts of evenness do not consider the identification of species, only the numbers of species and individuals.

We conclude that the abundance or number of species of annelids, echinoderms and arthropods in our laboratory or field communities was significantly less in aquaria contaminated with 177 μg/g creosote than in control aquaria. Mollusks were more resistant, but were significantly affected by 844 μg/g creosote in the field and by 4,420 μg/g in the laboratory. Indices of diversity and dominance indicated that communities in aquaria contaminated with 844 μg/g creosote and 4,420 μg/g differed from the control, but communities in aquaria containing 177 μg/g and in control aquaria were similar.

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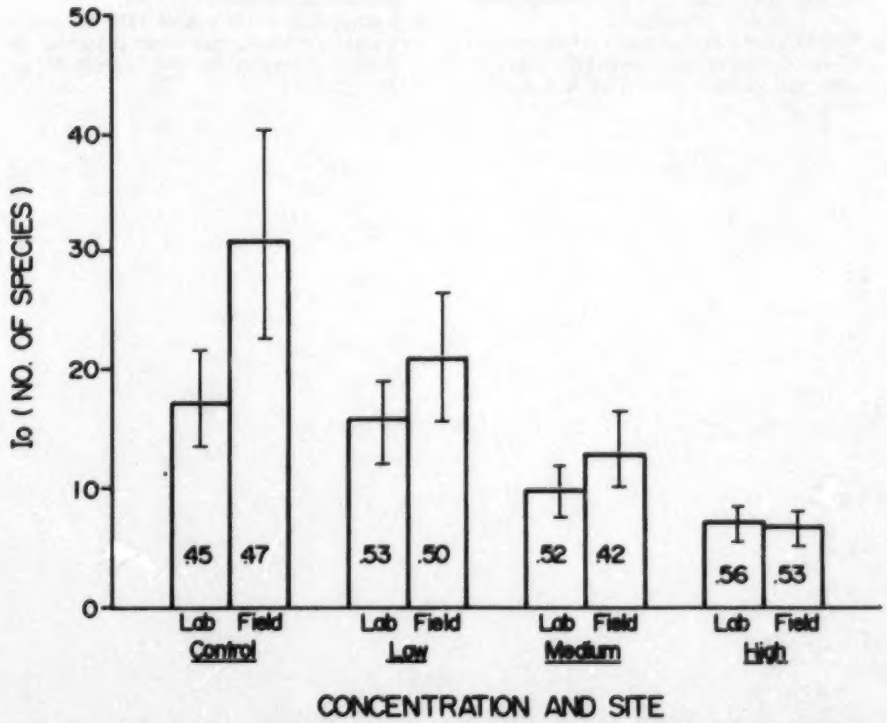

Fig. 3. Rarefaction indices generated from numbers of individuals and species collected from control aquaria and from aquaria contaminated with low (177 μg/g), medium (884 μg/g) and high (4,420 μg/g) concentrations of creosote. Io, average height of rarefaction curve with upper and lower bounds, number in each bar is the index of evenness of distribution of individuals within species.

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